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Crawl spaces as reservoirs for transmission of mold to the livable part of the home environment

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Abstract

Background: Recent studies suggest that exposure to mold in damp buildings is an important environmental risk factor for childhood respiratory illness. One potential source of a damp home, is crawl space construction. A poorly constructed crawl space not only presents the possibility of contributing to a 'damp' home but can also become a reservoir for fungal growth.
Objectives: Fungal levels in the livable indoor environment have been characterized in other studies, but little has been done to assess the potential for mold growth in the crawl space. This study examines the potential for mold growth and subsequent transmission from the crawl space into the home environment.
Methods: In this study, we assessed mold contamination levels within crawl spaces from 238 study homes in North Carolina. We determined whether air leakage from the heating, ventilation, and air conditioning (HVAC) system and associated ductwork, transmitted viable mold spores from the crawl space into the living spaces within the home.
Results: The results indicate that 19% of the homes demonstrated transmission of mold spores from the crawl space into the indoor environment, 45% of the homes displayed no transmission, and 36% of the homes were indeterminate.
Conclusions: The results support the hypothesis that the HVAC system can serve as a conduit for the transmission of mold spores from the crawl space to the indoor environment of a home. This transmission likely affects children's health, given the significant amount of time they spend in the home environment. For low-income families, the HVAC system may contribute an additional source of childhood exposure and highlights the importance of the assessment of indoor environmental hazards.

Keywords: allergy and asthma; bioaerosols; children's environmental health; exposure; housing; indoor air quality; molds and fungi.

Introduction

Crawl spaces represent a dominant construction type in the southeastern United States (US) due to their inexpensive nature and ease of construction. These advantages have led to the construction of over 9 million homes with crawl spaces in the southeastern US (1). Unfortunately, crawl spaces can also cause such problems as moisture condensation during the warmer months of the year (2). The average relative humidity (RH) in a crawl space during the warmer months can reach 85%–95% (3). Condensation can then form in the crawl space as a result of the high RH, thus creating an environment conducive to fungal growth. Fungal levels in crawl spaces can be amplified by as much as an order of magnitude in comparison with indoor air (4).

Mounting evidence suggests that exposure to indoor airborne mold is a risk factor for childhood respiratory illness (5). Dampness and mold in the home have been associated with multiple respiratory problems and allergies (6). At least 20% of US homes are estimated to have visible mold or dampness problems (7). Leakages from the crawl space into the living space of a home, which increases exposure to contaminants for residents, have also been documented (4). One potential consequence of fungal spore transmission from the crawl space into the livable area of the home is an increased risk of allergies and related illness (8–12). Despite the clear potential for crawl spaces to serve as a reservoir for fungal growth, little work has investigated the direct transmission of fungal spores from the crawl space to the interior environment through the heating, ventilation, and air conditioning (HVAC) system, or other systems that connect the crawl space with the livable part of the home environment.

Because children spend approximately 90% of their time indoors, with much of that time at home (13), assessing environmental risk in the home is important for delineating sources of exposure (14, 15). Children in unsafe or poor neighborhoods, are more likely to spend greater amounts of time indoors. In an initial limited pilot study of 10 homes, we observed that (a) the crawl space can serve as a reservoir for mold, and (b) a significant link exists between mold growth in the crawl space and transmission of that mold into the living spaces in the home

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(16). In this study, we designed a sampling protocol to answer two questions. First, does the crawl space serve as a reservoir for mold? Second, does the HVAC system serve as a conduit for mold spore transmission into the home?

Methods

Study population

We recruited the study participants using a Geographic Information System that identified potential residential parcels in Durham, Orange, Wilson, Wayne, and New Hanover Counties in North Carolina. These counties span the Piedmont, Central Coastal Plain, and the Coast in North Carolina (Figure 1). We identified willing participants through a letter-based recruitment program. To qualify for this study, four distinct criteria were required: i) the home had a crawl space, ii) the home had an HVAC system, iii) the HVAC system with the associated ductwork and air handler had to be located in the crawl space, and iv) the participant had to be willing to turn off the HVAC system for at least 4 h before the sampling appointment. We obtained informed consent from 238 participants according to a research protocol approved by the Institutional Review Board at Duke University. We collected interior and exterior bioaerosol samples to culture for respirable fungal spores in 238 homes to assess the concentration of aerosolized fungal spores.

Sampling method

Two trained indoor air quality technicians conducted the sampling using Andersen two-stage cascade impactors (ThermoFisher Scientific, Waltham, MA, USA formerly Andersen, Atlanta, GA, USA) that collect and separate both non-respirable and respirable size particles. The Andersen was connected to a vacuum pump calibrated to collect air samples at a rate of one cubic foot per min. We calibrated the equipment at the beginning of the day and again at the end of the day. Between each sample, we cleaned the Anderson using sterile pads saturated with 70% isopropyl alcohol. The sampling period for the outdoor sample, and all samples inside the home, was 3.5 min. The sampling period for all crawl space samples was 1.0 min. We used Malt Extract Agar (MEA), an aciduric mycologic medium designed for the collection of environmental fungi, as the collection medium for the impaction of fungal spores. After sampling, the culture plates were transported to our microbiology laboratory at Duke University, and incubated at ambient temperature for 96 h before enumeration and identification. The samples were protected from temperature extremes during transportation by using an electrically controlled cooler. Fungal identification was accomplished by macroscopic examination of colony morphology and microscopic examination of fungal elements. Identification was accomplished in part by using *Medically Important Fungi* (17). The colony forming unit (CFU) counts were then converted using the positive hole correction method (18).

Data collection

Table 1 summarizes the locations sampled at each home. We conducted Level 1 sampling in all 238 homes. In 147 of the Level 1 homes, we also conducted Level 2 sampling, and in 45 of the Level 2 homes, we conducted Level 3 sampling. Thus, we have a nested study design in which the Level 3 sample homes are a subset of the Level 2 sample homes, which are, in turn, a subset of the Level 1 sample homes. Depending on the phase of the research project and housing characteristics, we collected either five samples (Level 1 collecting O, $I_{BASE,}$ CS_{ACC}, I_{ACC}, D_{ACC}; n=238) or eight samples (Level 2 collecting O, I_{BASE}, CS_{BASE}, I_{HVAC}, D_{HVAC}, CS_{ACC}, I_{ACC}, D_{ACC}; n=147). For all homes, we collected the samples in the order listed in Table 1. The layout of a general crawl space HVAC system is depicted in Figure 2, which demonstrates several features of the crawl space environment that are important to this study. First, as required for eligibility, the ductwork for the HVAC runs through the crawl space, meaning that any small holes or gaps in the ductwork provide an opening through which fungal spores circulating in the crawl space can be drawn into the HVAC system and transmitted to the interior of the home. The triangle in Figure 2 represents the air near the return vent, which is a good composite measure of overall air quality in the home, combining air from a variety of sources, including HVAC air, leaks from crawl space air, and leaks from outdoors. Indoor samples were collected at this location in all homes. Further, the concentric circles symbol in Figure 2 corresponds to the first diffuser vent off of the HVAC system; air sampled at this location inside the home provides a direct measure of the contribution of fungal spores from HVAC air (and associated crawl space impacts) to interior air quality.

Level 1 sampling included a total of five samples (see Table 1). We collected the first sample, referred to as the outdoor (O) sample, outside the house. Before turning on the HVAC system, we collected the indoor baseline (I_{BASE}) sample, inside the house near the 'return'

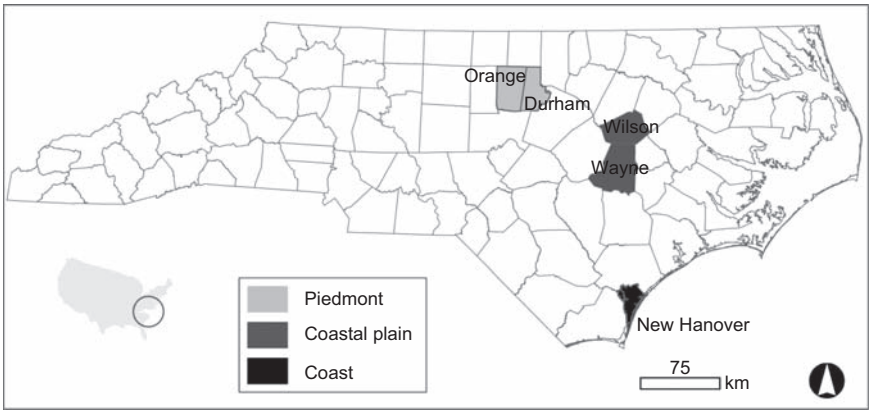

Figure 1 Study locations in North Carolina.

Table 1 Bioaerosol sampling strategy.

Code	Location	HVAC status	Location description and crawl space sampling technique	Level 1	Level 2
				5 Samples	8 Samples
O	Outdoor	N/A	Near most commonly used door, as identified by resident	X	X
I_{BASE}	Indoor	Off 4+ h	Near the return vent for the HVAC system	X	X
CS_{BASE}	Crawl space	Off 4+ h	Crawl space distal sampling technique collected at entry		X
I_{HVAC}	Indoor	On >5 min	Near the return vent for the HVAC system		X
D_{HVAC}	Diffuser	On >5 min	From the first diffuser that comes off the HVAC system		X
CS_{ACC}	Crawl space	On	Crawl space accessed, sample collected near the first drop off the HVAC system	X	X
I_{ACC}	Indoor	On	Near the return vent for the HVAC system; >5 min after CS_{ACC} sample	X	X
D_{ACC}	Diffuser	On	From the first diffuser that comes off the HVAC system; >5 min after CS_{ACC} sample	X	X
n				238	147

HVAC, heating and air conditioning system.

vent for the HVAC system (see Figure 2). A third sample was taken by physically accessing the crawl space (CS_{ACC}). Close to the first drop from the air handling unit in the crawl space, the Andersen was held at least one foot off the ground to prevent inadvertent disruption once the vacuum was turned on. After this crawl space sample was collected and we exited the crawl space, the HVAC system fan was allowed to run for 5 min. Two more interior samples were taken, one again near the return grill (I_{ACC}) and one at a supply air diffuser (D_{ACC}). The D_{ACC} sample was collected inside a short polyethylene tube that was temporarily taped around the supply register (see Figure 3). This tube isolated the supply air from potential contaminant sources within the house, thus allowing characterization of the relative contribution of the HVAC system to the total fungal spore accumulation.

In an effort to expand our ability to distinguish the effects of turning on the HVAC system alone vs. accessing the crawl space, we conducted Level 2 sampling. In addition to the five samples collected as in Level 1, Level 2 sampling included 3 new samples, all three of which occurred after collecting the indoor baseline (I_{BASE}) sample and before physically accessing the crawl space. In homes in which the crawl space had not been entered on the day of sampling and the design of the entry to the crawl space allowed, we used a non-invasive distal sampling technique, with a tray attached to the end of a telescoping pole, to collect a baseline crawl space sample (CS_{BASE}). The pole was inserted into the crawl space without touching any surfaces in the crawl space, to avoid disturbing any settled spores. The HVAC system was turned on after collecting the CS_{BASE} sample. After the HVAC system fan was allowed to run for at least 5 min, two additional indoor samples were taken, one near the return grill (I_{HVAC}) and one at a supply air diffuser or register (D_{HVAC}).

Transmission determination

To assess the possibility of mold transmission from the crawl space into the home, we classified homes into three categories: transmission

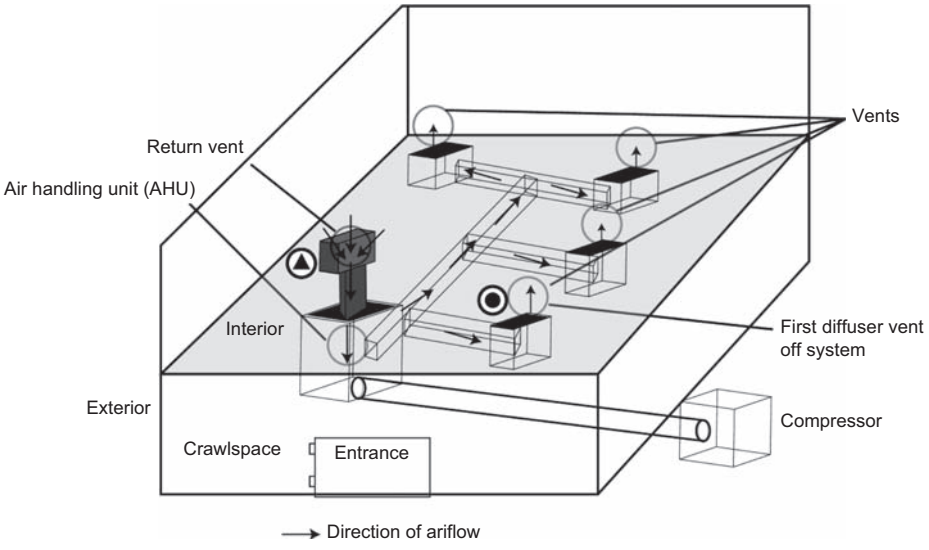

Figure 2 Simplified layout of t HVAC system in a crawl space. Triangle symbol indicates location of indoor samples (I_{BASE}, I_{HVAC}, I_{ACC}); concentric circle symbol indicates location of the diffuser samples (D_{HVAC} and D_{ACC}).

Figure 3 Diffuser sampling method.

(T), non-transmission (NT), or indeterminate transmission (IT). We developed the classification based on the levels, composition, and rank order of mold in the bioaerosol samples both before and after the HVAC system was turned on, and both before and after the crawl space was physically accessed. In particular, we evaluated whether indoor bioaerosol levels increased after the HVAC system was turned on, and how the composition of the fungal spores in the second indoor sample (I_{HVAC}) compared with the composition in the baseline indoor sample (I_{BASE}), the outdoor (O), and the initial baseline distal crawl space (CS_{BASE}) samples. In many cases, differences in species composition allowed us to distinguish between changes in the indoor samples ($I_{HVAC} - I_{BASE}$) that were consistent with pulling in outdoor air vs. pulling in air from the crawl space. A similar analysis was undertaken for the final indoor samples (I_{ACC}). In addition, the diffuser samples allowed us to draw distinctions between mold spores being transferred from the crawl space vs. mold growing in the HVAC system itself. Homes having an obvious link between the crawl space and indoor bioaerosol levels, composition, and/or rank order, were categorized as transmission. Those that did not exhibit any link between the crawl space and the indoor samples were classified as non-transmission. Indeterminate homes had some indicators of crawl space transmission, but could not be distinguished from the possibility of mold growing in the home or the HVAC system or the HVAC system pulling in outdoor air. For each home, classification was determined independently by two investigators (M.L.M. and W.R.T.). Then, the differences in classification were resolved in a research conference. The classification system is perhaps best explained by looking at the three specific examples presented in Table 2. In the transmission home, the initial indoor sample was low (I_{BASE}=522 CFU/m^3; after the HVAC system had been turned off for at least four hours). The non-invasive crawl space sample was relatively high (CS_{BASE}=12,831 CFU/m^3). After turning on the HVAC system, the indoor sample increased, and the diffuser sample was higher than the first indoor sample as well (I_{HVAC}=1,288 CFU/m^3 and D_{HVAC}=1,022 CFU/m^3). After invasive sampling, the crawl space levels more than tripled (CS_{ACC}=41,146 CFU/m^3), and the indoor and diffuser samples increased by an order of magnitude. Both the total levels of colony forming units and the particular amplification of *Penicillium* – which was abundant in the crawl space but quite low in the outdoor sample – indicate that transmission of mold spores from the crawl space to the livable part of the home environment was occurring. (Note that we speciated many types of spores, but for space reasons, we list only *Penicillium* and *Cladosporium* levels in Table 2.) We conclude that for this home, simply turning on the HVAC transmits mold spores from the crawl space into the livable part of the home environment. The transmission is substantially amplified by disturbing the crawl space environment via invasive sampling. In contrast, in the non-transmission home, the indoor levels remained quite stable from the beginning of the sampling protocol through the end, including those collected after the resuspension of spores during the invasive sampling of the crawl space. In the indeterminate home, the indoor levels increased over each subsequent stage of the sampling protocol, but the particular type of spores that were amplified did not match up well with the dominant spores in the crawl space samples.

Statistical methods

We used statistical methods to confirm the validity of our somewhat qualitative transmission classification method. For all homes, we compared I_{BASE} and I_{ACC} fungal counts using a paired sample t-test

Table 2 Example of sample counts for one home in each transmission classification reported as colony forming units per cubic meter (CFUs/m^3).

Sample	Transmission			Non-transmission			Indeterminate transmission		
	Total	*PEN*	*CLAD*	Total	*PEN*	*CLAD*	Total	*PEN*	*CLAD*
O	699	178	290	940	71	188	11,756	134	278
I_{BASE}	522	71	373	373	92	30	459	51	40
CS_{BASE}	12,831	623	6682	3346	214	105	11,813	1390	700
I_{HVAC}	1288	256	643	472	146	61	630	71	71
D_{HVAC}	1022	302	373	536	210	20	972	146	146
CS_{ACC}	41,146	7868	2447	41,146	22,540	70	15,778	434	249
I_{ACC}	11,756	2218	723	459	188	40	1251	146	200
D_{ACC}	11,756	2896	497	892	222	51	1642	134	256

PEN, *penicillium*; *CLAD*, *cladosporium*.

within each transmission grouping. Significant increases from the I_{BASE} to the I_{ACC} fungal counts, are a major indication of fungal spore transmission from the crawl space. We also used t-tests for paired samples to test for differences across samples by transmission category, for the subset of homes for which we were able to collect eight samples (Level 1 plus Level 2 sampling). We compared I_{BASE} to I_{HVAC} and I_{ACC}, D_{HVAC} to D_{ACC}, and CS_{BASE} to CS_{ACC}. As the eight-sample homes have three indoor samples each, we adjusted our alpha value to 0.017 for the indoor samples, to reduce the likelihood of a Type I error. For the indeterminate transmission category, we split this group into two: those homes in which *Penicillium* may be growing in the HVAC system and those in which it is not likely to grow. Analyzing the indeterminate transmission homes within this context is important because this condition represented a major reason why we could not distinguish between a transmission home and an indeterminate transmission home with mold growing in the HVAC system. We also report the correlations between fungal counts before and after turning on the HVAC system and accessing the crawl space. For all statistical analyses we used Stata/SE for Windows version 9.0 (StataCorp LP, College Station, TX, USA) and an *alpha* value of 0.05 unless otherwise stated.

Results

Study population and fungal spore transmission

We sampled 238 homes for this study, of which 147 were eight-sample, Level 2 homes. For all homes, we classified 108 as non-transmission homes, 45 as transmission homes, and 85 as indeterminate transmission homes. Tables 3 and 4 provide a summary of the total fungal levels in all homes and in Level 2 homes, respectively.

Table 3 Summary statistics for all homes, per transmission category (n=238).

Classification	Sample	n	Mean	SD	Min	Max
Non-transmission	O	108	3013	3850	51	11,756
Homes	I_{BASE}	108	1184	2267	30	11,756
	CS_{ACC}	108	17,633	16,219	105	41,146
	I_{ACC}	108	658	498	82	3430
	D_{ACC}	108	680	1576	10	11,756
CS-transmission	O	45	2677	3514	278	11,756
Homes	I_{BASE}	45	1243	2403	82	11,756
	CS_{ACC}	45	34,667	13,298	2909	41,146
	I_{ACC}	45	3162	3631	222	11,756
	D_{ACC}	45	3393	3825	313	11,756
Indeterminate	O	64	2143	2983	200	11,756
Transmission	I_{BASE}	64	1109	1620	113	11,756
Homes with	CS_{ACC}	64	29,642	15,727	1519	41,146
Penicillium	I_{ACC}	64	1937	2487	373	11,756
	D_{ACC}	64	1691	2488	146	11,756
Indeterminate	O	21	3247	4300	422	11,756
Transmission	I_{BASE}	21	1826	2718	222	11,756
Homes without	CS_{ACC}	21	31,550	15,749	2496	41,146
Penicillium	I_{ACC}	21	2588	3360	472	11,756
	D_{ACC}	21	1960	2650	361	11,756

O, sample collected outdoors; CS_{ACC}, sample collected in crawl space at the first drop of air handling unit; I_{BASE}, sample collected indoors prior to turning on HVAC; I_{ACC}, sample collected indoors after crawl space sampling at the drop of the air handling unit; D_{ACC}, sample collected at diffuser after invasive crawl space sampling.

All homes, n=238 Our analysis supports the classifications determined above. Homes in the transmission category showed a significant increase in indoor total fungal counts after the crawl space was disturbed by invasive sampling, p=.001 (Figure 4). Such an increase would potentially also result from any other similar disturbance. On the other hand, non-transmission homes showed a significant decrease, p=.0083, between the indoor base sample and the sample after the crawl space was accessed via invasive sampling, likely indicating a filtering role for the HVAC system. For the

Table 4 Summary statistics for homes with level 1+ level 2 sampling, per transmission category (n=147).

Classification	Sample	n	Mean	SD	Min	Max
Non-transmission	O	58	2511	3239	267	11,756
Homes	I_{BASE}	58	1410	2647	71	11,756
	CS_{BASE}	58	10,154	13,004	434	41,146
	I_{HVAC}	58	854	736	0	4508
	D_{HVAC}	58	771	1516	71	11,756
	CS_{ACC}	58	22,201	15,886	735	41,146
	I_{ACC}	58	679	438	124	2248
	D_{ACC}	58	956	2098	82	11,756
Transmission	O	37	2716	3514	278	11,756
Homes	I_{BASE}	37	1328	2581	82	11,756
	CS_{BASE}	37	13,631	15,244	249	41,146
	I_{HVAC}	37	1212	1854	178	11,756
	D_{HVAC}	37	875	890	92	4606
	CS_{ACC}	37	36,312	11,272	3815	41,146
	I_{ACC}	37	3490	3855	222	11,756
	D_{ACC}	37	3666	3806	510	11,756
Indeterminate	O	36	1393	937	200	4414
Transmission	I_{BASE}	36	1075	1048	113	6204
Homes with	CS_{BASE}	36	15,703	15,122	935	41,146
Penicillium	I_{HVAC}	36	2263	2683	278	11,756
	D_{HVAC}	36	1827	2583	200	11,756
	CS_{ACC}	36	31,906	14,053	5208	41,146
	I_{ACC}	36	1774	2007	373	11,756
	D_{ACC}	36	1919	2690	325	11,756
Indeterminate	O	16	2487	3722	422	11,756
Transmission	I_{BASE}	16	1098	1345	222	5802
Homes without	CS_{BASE}	16	11,122	14,557	935	41,146
Penicillium	I_{HVAC}	16	2457	3756	313	11,756
	D_{HVAC}	16	1833	3208	146	11,756
	CS_{ACC}	16	30,967	15,796	2961	41,146
	I_{ACC}	16	2068	2854	472	11,756
	D_{ACC}	16	1616	1586	361	6708

O, sample collected outdoors; CS_{BASE}, sample collected in crawl space using non-invasive technique; CS_{ACC}, sample collected in crawl space using invasive technique; I_{BASE}, sample collected indoors prior to turning on HVAC; I_{HVAC}, sample collected indoors after non-invasive crawl space sampling; I_{ACC}, sample collected indoors after invasive crawl space sampling; D_{HVAC}, sample collected at diffuser after non-invasive crawl space sampling; D_{ACC}, sample collected at diffuser after invasive crawl space sampling.

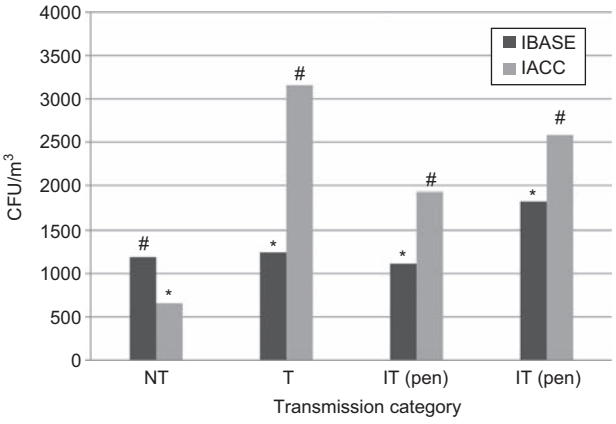

Figure 4 Mean indoor sample fungal counts by transmission category, all homes (columns with different symbols, * vs. #, represent significant differences across samples, p<.05).

indeterminate transmission homes, we observed a significant increase in indoor total fungal counts in homes in which *Penicillium* may be growing in the HVAC system (n=64). In the remaining indeterminate transmission homes, although based on a small sample size (n=21), a significant increase in indoor total fungal counts was also noted, p=.04.

Level 2 homes, n=147 Analysis of indoor fungal counts for the eight sample homes provided further confirmation of the transmission classifications. Homes classified as non-transmission showed no significant increase across the fungal counts in the three indoor samples (Figure 5). In contrast, transmission homes showed a dramatic and statistically significant increase between the indoor samples collected before the invasive crawl space sample (I_{BASE} and I_{HVAC}) and the indoor sample collected after the crawl space was accessed for invasive sampling (I_{ACC}). The difference between non-transmission and transmission homes existed, despite a statistically significant increase in the crawl space fungal counts in both groups, between the non-invasive sampling and accessing the crawl space (Figure 6). This finding provides strong evidence that resuspension of fungal spores in the crawl space, through entry into the crawl space, can be rapidly transmitted into the home interior. The correlation coefficients between samples collected after turning on the HVAC and after accessing the crawl space (Table 5) support these observations. For non-transmission homes, the correlation coefficients between I_{HVAC} and I_{ACC} and between D_{HVAC} and $D_{ACC,}$ are both relatively high at 0.74, indicating that accessing the crawl space does not greatly alter the fungal spore levels in the livable part of the home environment or at the diffuser. For transmission homes, the coefficient is lower (0.43) between I_{HVAC} and I_{ACC}, with an even smaller correlation coefficient between D_{HVAC} and D_{ACC} (0.11), indicating that accessing the crawl space changes the levels of fungal spores entering the transmission homes. This observation, coupled with the statistically significant increase in mean indoor and diffuser fungal spore levels (Figure 7)**,** supports the conclusion that the resuspension of fungal spores upon accessing the crawl space results in increased transmission. Additionally, the correlation between the I_{ACC} and the D_{ACC} samples is much higher in transmission homes, at 0.82, when compared with non-transmission homes at 0.47, indicating that the fungal spore levels throughout the house are consistently elevated after accessing the crawl space and could result in transmission via the diffuser. These samples were the only indoor samples with mean counts >3400 CFU/m^3, almost 1000 CFU/m^3 greater than the closest indoor mean.

The results from the indeterminate transmission analyses suggest that, in these two groups of homes, the pressure gradient created by turning on the HVAC system is enough to trigger transmission either from the HVAC system itself, or from another source. Both indeterminate transmission groups, like the non-transmission and transmission groups, had significant

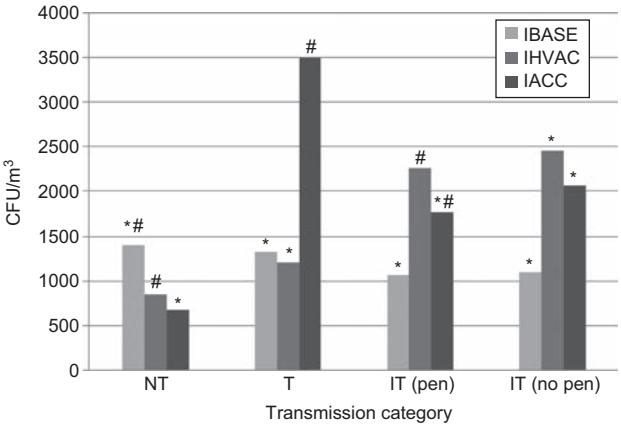

Figure 5 Mean indoor sample fungal counts by transmission category, Level 2 homes (columns with different symbols, * vs. #, represent significant differences across samples, p<0.017).

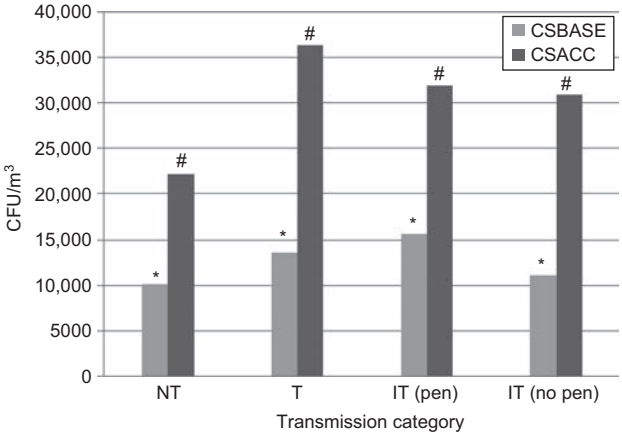

Figure 6 Comparison of mean fungal counts in crawl space samples by transmission category, 8-sample homes (columns with different symbols, * vs. #, represent significant differences across samples, p<.05).

Table 5 Correlations between indoor, diffuser, and crawl space samples before and after turning on the HVAC and accessing the crawl space.

Level 2	All	NT	T	IT (*Pen*)	IT (no *Pen*)
$I_{BASE} - I_{HVAC}$	0.33	0.66	0.67	0.12	0.58
$I_{HVAC} - I_{ACC}$	0.52	0.74	0.43	0.84	0.74
$I_{BASE} - I_{ACC}$	0.23	0.40	0.28	0.27	0.88
$CS_{HVAC} - CS_{ACC}$	0.27	0.27	0.28	0.16	0.36
$D_{HVAC} - D_{ACC}$	0.42	0.74	0.11	0.62	0.72
$I_{HVAC} - D_{HVAC}$	0.70	0.49	0.09	0.93	0.72
$I_{ACC} - D_{ACC}$	0.72	0.47	0.82	0.84	0.17

Pen, penicillium.

increases in crawl space fungal counts between CS_{BASE} and CS_{ACC}, but this change did not influence fungal counts at the diffuser as there was no significant change between D_{HVAC} and D_{ACC} (Figure 7). The mean fungal spore counts at the diffuser for both of these groups were already elevated (almost twice the levels in the non-transmission and transmission homes) before the crawl space was accessed and could have resulted from simply turning on the HVAC system, which is suggested by the changes in mean fungal counts in the indoor samples. In cases where *Penicillium* may be growing in the HVAC system, potentially in areas where water condenses, mean fungal spore counts for I_{BASE} and I_{HVAC} were significantly different (p=.014), more than doubling (Figure 5). The mean counts between I_{HVAC} and I_{ACC} were not significantly different (p=.11) and actually drop, again potentially indicating some filtering effect by the HVAC. All the results taken together suggest that just turning on the HVAC system (and the resulting pressure differential) in these homes results in increased transmission of fungal spores because the fungal counts are higher in I_{HVAC} than in I_{BASE}, whereas the disturbance created by accessing the crawl space did not result in an increase between I_{HVAC} and I_{ACC}. For indeterminate transmission homes in which *Penicillium* is not likely growing in the HVAC system, fungal counts after turning on the HVAC system again more than doubled, although the difference was not statistically significant, likely due to the relatively small sample size (n=16). The indeterminate transmission group without *Penicillium* also showed a small, but not statistically significant drop in mean counts between I_{HVAC} and I_{ACC}. The correlation coefficients again support these results. The correlation coefficient between the I_{BASE} and I_{HVAC} for indeterminate transmission homes with *Penicillium,* is only 0.12, indicating that turning on the HVAC can alter indoor fungal spore counts in a manner that is not related to the baseline counts, but accessing the crawl space has little influence as the correlation between I_{HVAC} and I_{ACC} is remarkably high at 0.84 with little change in the mean levels. Similarly, the influence of D_{HVAC} on I_{HVAC} and D_{ACC} on I_{ACC} is also consistent with transmission via the HVAC, resulting in correlation coefficients of 0.93 and 0.84, respectively. Indeterminate transmission homes without *Penicillium* do not show such a high correlation between these samples, having coefficients of 0.72 and 0.17, respectively, indicating that the diffuser is not the only route of fungal spore entry into the home environment for these homes.

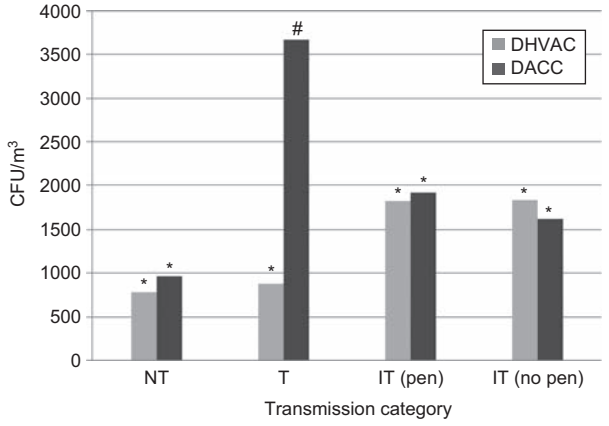

Figure 7 Comparison of mean fungal counts in diffuser samples by transmission category, 8-sample homes (columns with different symbols, * vs. #, represent significant differences across samples, p<0.05).

CS_{ACC} samples in both indeterminate transmission groupings, appear to be intermediates between the transmission and non-transmission homes. Indeterminate transmission homes with *Penicillium* possibly growing in the HVAC system, also appear to have slightly higher levels of fungal growth in the crawl space than the ones where this is unlikely. Another important feature of the crawl space fungal levels is that their magnitude is substantially greater than that generally observed in the indoor and diffuser samples, indicating that the physical structure of the crawl spaces may be helping to elevate concentrations of mold spores. For the diffuser samples, only the transmission homes demonstrate a significant increase in fungal levels after crawl space disturbance (Figure 7).

Monitoring air leakage between various parts of the home helps determine the vehicle for transport of air and airborne contaminants from the crawl space into the livable parts of the home environment. The results of the leakage analysis conducted in 45 homes are detailed elsewhere (1). Here, we provide a brief summary of the results. Three leakage paths were measured: total house air leakage, air leakage between the living space and the crawl space, and air leakage between the duct work and crawl space. Using classifications based on cubic feet per minute at 50 Pascals (CFM 50) per square foot of surface area, 13% had major leakage, 20% had excessive leakage, 42% had moderate leakage, 24% had limited leakage, and zero had minimal leakage.

Crawl space-to-house air leakage quantifies the amount of air exchange resulting from holes in the floor between the crawl space and the house. The majority of homes (69%) had between 11% and 30% of the total house air leakage coming from the crawl space. Air leakage in crawl space ducts represents the amount of air in CFM passing through holes or gaps in the ductwork. Using classifications based on cubic feet per minute at 25 Pascals (CFM 25) per square foot of floor area, 65% had major duct leakage, 18% had excessive leakage, 9%

had moderate leakage, 4% had limited leakage, and zero had minimal leakage. Five homes were not classified because they were unable to reach their target pressure.

Discussion and conclusions

Our sampling protocol was developed to evaluate the potential for mold growth in a crawl space, and the subsequent transmission of fungal spores into the livable home environment. The protocol was also developed to simulate the disturbances that might occur when a strong wind or other disruption enters the crawl space and disturbs settled spores.

The eight sample homes were the ideal measure for investigating these issues. These homes offered the ability to sample in a non-invasive way, which simulated the undisturbed crawl space environment. The homes also offered the chance to take baseline indoor measures with the HVAC running, which gave us the opportunity to look at the contribution of the HVAC system to the overall indoor fungal count. Of the eight sample homes that we tested, 25% were determined to be transmission homes.

This analysis indicates that the crawl space is a significant potential reservoir for the amplification of mold. The HVAC system clearly served as a vehicle for the transmission of this contamination from the crawl space into the living spaces of 19% of the houses sampled. In only 45% of the homes were we able to rule out transmission entirely. Based on our observations, the potential causes of the mold growth and spore distribution appear to be inadequate insulation of cold surfaces in the crawl space, leading to extensive condensation. In addition, the leakage of air-conditioned air from supply ducts appeared to have caused extensive condensation from the moist, warm outdoor air that infiltrates the crawl space.

The project design did have limitations that affected our ability to examine the influence of simply turning on the HVAC system on indoor fungal spore exposure. The structure of some homes did not permit technicians to collect a non-invasive crawl space sample in all homes, if the equipment could not be placed without disturbing part of the crawl space. In addition, collecting an indeterminate crawl space sample after the HVAC was turned on, but before the crawl space was accessed, would provide information about the effect of the pressure differential created by the HVAC on circulating fungal spores in the crawl space that might be transmitted to the home environment.

Beyond the design of the project, the lower fungal levels in the CS_{BASE} samples of the non-transmission homes suggest that part of the reason for decreased likelihood in these homes may relate to a lower level of fungal growth in the crawl spaces to begin with. This low-level may result from different construction types, local environmental factors, or age of housing.

Overall, this work highlights the important role that crawl space construction may play with respect to respirable bioaerosols in the home environment and childhood exposure. In some homes, pressure gradients created by the HVAC system itself may contribute to transport of mold from the crawl space to the home environment. In addition, human, animal, and weather-related disturbances of the crawl space may stir up fungal spores, thus facilitating their transmission into the home environment. Our results point to the importance of sealing leakages between the crawl space and the home interior to avoid the potential transmission of serious allergen and asthma triggers. Nonetheless, transmission via the HVAC system remains possible. Homes with asthma or allergy sufferers should take necessary precautions to limit the disturbance of crawl spaces and subsequent transmission of bioaerosol indoors. One potential strategy would be to turn off the HVAC before accessing the crawl space, and leave the system off for a period of time following such access to allow suspended spores to settle before turning the system back on, thereby reducing the likelihood of transmission.

Although transmission was found regardless of home age, this exposure contributes to the cycle of disadvantage and disability for low-income families. For more advantaged families, this risk is important and potentially malleable with education and precautions. For disadvantaged families, this risk accumulates with other risks, increasing the respiratory impact. Children, especially those in low-income and unsafe neighborhoods, spend most of their time indoors. This study highlights the importance of the assessment of indoor environmental hazards, if we are to create protective environments for all children in our communities.

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